Diss. ETH No. 18651

Altered signaling at glutamatergic synapses: Behavioural and neuroanatomical implications of (global and hippocampus-specific) SynGAP knockout.

A dissertation submitted to the
ETH ZURICH
For the degree of
Doctor of Sciences

Presented by

Mary Muhia

MRes in Experimental Neuroscience,
Imperial college, University of London

Born, 23rd October, 1980

Kenyan Citizen

Accepted on recommendation of
Prof. Dr. Joram Feldon, examiner
Dr. Irene Knuesel, examiner
Prof. Dr. Jean-Marc Fritschy, co-examiner
Prof. Dr. Aline Marighetto, co-examiner

2009

SUMMARY

The brain-specific synaptic Ras/Rap guanosine triphosphate (GTPase)-activating protein (SynGAP) regulates two distinct intracellular signaling events in response to *N*-methyl-D-aspartate receptor (NMDAR) activation. SynGAP's biochemical and physiological function is well established. Notwithstanding, evidence for the functional relevance of SynGAP to behavioural function is very limited.

The main objective of this thesis was to evaluate the impact of decreased SynGAP expression (knockout) on NMDAR-dependent behavioural functions, as well as to assess its potential to mimic schizophrenia-related behavioural functions associated with NMDAR dysfunction. To address this, the thesis was designed to carry out a comprehensive behavioural and neuroanatomical characterization by utilising two distinct transgenic SynGAP knockout mouse lines: (i) SynGAP heterozygous knockout ($SG^{+/-}$) mice, in which approximately half of the SynGAP protein is constitutively missing in the brain, (ii) SynGAP homozygous conditional knockout ($SG^{flox/flox}$) mice, in which the *syngap* gene harbours two *loxP* sites that were excised selectively in the adult hippocampus by means of Cre recombinase-expressing recombinant adeno-associated viral (rAAV) vectors.

The first series of experiments illustrated that constitutive heterozygous SynGAP knockout can lead to behavioural as well as morphological phenotypes indicative of hippocampal and NMDAR dysfunction (Chapter 2). When examined separately, spatial reference memory function was largely intact in $SG^{+/-}$ mice, yet a severe deficit emerged following a concurrent evaluation of reference and working memory function. This suggested of increased vulnerability to increasing memory load following this manipulation.The absence of a deficit in the non-spatial object recognition memory indicated that SynGAP may be more relevant to spatial memory function. At the same time, the results obtained here illustrated that the impact of this genetic manipulation translated beyond mnemonic function given the robust observations of hyperactivity and anxiolytic traits. Furthermore, the behavioural phenotype was paralleled by severe morphological changes involving a substantial decrease in hippocampal GABAergic cells and decreased adult dentate gyrus neurogenesis, possibly contributing to the subsequent emergence of the behavioural phenotype. Hence, the results obtained here were indicative of a functional role of SynGAP in specific cognitive and non-cognitive functions that are sensitive to NMDAR function.

The next series of experiments examined further the behavioural impact of heterozygous SynGAP knockout ($SG^{+/-}$ mice), and its potential to mimic schizophrenia-related behavioural functions associated with NMDAR dysfunction (Chapter 3). SynGAP heterozygous knockout mice were spontaneously hyperactive, and displayed weaker

locomotor habituation over time. Systemic NMDAR blockade by the non-competitive NMDAR antagonist, MK-801 further exacerbated this hyperactivity phenotype. In contrast, the motor stimulating effect to the indirect dopamine receptor agonist, amphetamine was attenuated in $SG^{+/-}$ mice, suggesting of dopamine hypofunction. Attentional function, as examined by the prepulse inhibition and latent inhibition paradigms was largely intact in $SG^{+/-}$ mice. However, $SG^{+/-}$ mice showed a pronounced deficit in preference for social novelty without affecting social seeking, consistent with the suggested link between NMDAR hypofunction and negative schizophrenia symptoms. Post-mortem analysis of brain neurochemistry did not reveal any significant changes in neurotransmitters and related metabolites content. Overall, the $SG^{+/-}$ mouse model captures specific schizophrenia endophenotypes, and might therefore be instrumental in elucidating the molecular control over specific schizophrenia-related abnormalities, in which NMDAR dysfunction is implicated.

The series of experiments that followed (Chapter 4) evaluated the consequences of heterozygous SynGAP knockout ($SG^{+/-}$ mice) in instrumental learning by assessing behavioural inhibition and temporal discrimination, functions known to critically depend on NMDAR integrity. The results revealed that response inhibition and timing behaviour were largely unaltered by heterozygous SynGAP deficiency. However, this mutation led to a significant increase in response vigour, with no indication of motivational changes. In addition, a deficit in the acquisition of extinction memory was shown by $SG^{+/-}$ mice, yet this appeared to be schedule-specific and could not be generalized to all the schedules used. In summary, the results showed that a constitutive reduction in SynGAP expression increased the vigour in the execution of learned operant behaviour without compromising its temporal control, thus only resembling some impact of NMDAR dysfunction.

The final study (Chapter 5) was aimed to examine the morphological and behavioural impact of adult hippocampus-specific SynGAP knockout using viral-mediated conditional deletion, thus allowing us to determine its role in a specific brain region and a further possibility to dissociate SynGAP's role during development from its influence on adult behaviour. A substantial decrease in hippocampus SynGAP expression led to a significant increase in NMDAR and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) expression levels, likely representing their enrichment at glutamatergic synaptic sites. Similarly, this manipulation led to a significant decrease in adult dentate gyrus neurogenesis. Behaviourally, hippocampus-specific SynGAP deficiency led to a deficit in spatial reference memory acquisition and a severe deficit in the acquisition and retention of reversal learning. Furthermore, this manipulation led to a delay-dependent working memory deficit and enhanced

spontaneous locomotor activity. On the other hand, anxiety-related behaviour was unimpaired following hippocampus-specific SynGAP knockout. These behavioural results partially reproduced the findings described in constitutive heterozygous SynGAP knockout mice, pointing to similarities as well as divergent roles of SynGAP during development and adult hippocampal functions.

Taken together, these findings provide a clear illustration of SynGAP's functional relevance to NMDAR function on behaviour. Furthermore they provide novel information on the functional significance of SynGAP during development and adulthood. As demonstrated above, not all NMDAR-dependent behavioural functions were altered by SynGAP knockout and the magnitude of severity is some phenotypes was less compared to that seen following a complete loss of NMDAR function. Hence, this can be taken to suggest that SynGAP loss might be sufficient to alter some but not all NMDAR-dependent behavioural processes. This view is not surprising given that SynGAP manipulation interferes with two distinct signaling cascades following NMDAR activation. A more severe phenotype would be expected following complete manipulations of the NMDAR because this would interfere with all the up- and downstream modulatory aspects that mediate NMDAR function. Nonetheless, SynGAP knockout can be considered to be instrumental in elucidating the mechanisms mediated by the NMDAR under physiological conditions, including the mechanisms of NMDAR dysfunction in pathological brain conditions.

SINTESI

La proteina sinaptica attivante la Ras/Rap guanosine trifosfato (GTPasi) (SynGAP), specifica del cervello, regola due eventi intracellulari distinti in risposta all'attivazione del recettore *N*-metil-D-aspartato (NMDAR). Le funzioni biochimiche e fisiologiche di SynGAP son ben definite. Tuttavia, mancano prove di una rilevanza funzionale di SynGAP nel controllo comportamentale.

L'obiettivo principale di questa tesi è stato di valutare l'impatto di una diminuita espressione di SynGAP (knockout) sulle funzioni comportamentali dipendenti da NMDAR, così come le sue potenzialità nel modellare comportamenti di tipo schizofrenico associati alla disfunzione di NMDAR. La presente tesi è stata ideata ai fini di una caratterizzazione comportamentale e neuroanatomica si due distinti ceppi murini SynGAP knock out: (i) SynGAP knockout eterozigoti ($SG^{+/-}$), in cui metà della proteina SynGAP è assente nel cervello, (ii) SynGAP conditional knockout ($SG^{flox/flox}$), in cui il gene *syngap* è fiancheggiato da due siti *loxP* , permettendo la delezione del gene nell'ippocampo adulto mediante un vettore ricombinante virale adeno-associato (rAAV) che esprime la recombinasi Cre.

La prima serie di esperimenti illustra come i topi eterozigoti per SynGAP presentino un fenotipo morfologico e comportamentale indicativo di una disfunzione ippocampale e NMDAR (Capitolo 2). I risultati hanno dimostrato un deficit a livello di memoria spaziale e di riconoscimento di oggetti. Tali deficit sono associati a alterazioni di tipo non cognitivo, quali iperattivo e ridotta ansietà. Inoltre, a livello neuro anatomico, sono stati osservati alterazioni morfologiche quali riduzione del numero di cellule GABAergiche e di neurogenesi nel giro dentato dell'ippocampo.

Nella successiva serie di esperimenti, è stato ulteriormente analizzato l'impatto comportamentale del knockout eterozigote SynGAP e le le sue potenzialità nel modellare comportamenti di tipo schizofrenico associati alla disfunzione di NMDAr (Capitolo 3). I topi mutanti presentano alterazioni nel pattern locomotorio, esacerbate dalla somministrazione dell'antagonista non-competitivo di NMDAR, MK-801. Al contrario, la risposta locomotoria all'amfetamina, agonista indiretto di recettori dopaminergici, si è mostrata ridotta. I deficit attenzionali sono stati valutati ,mediante i paradigmi di *prepulse* e *latent inhibition*. Nessuno dei due test ha rivelato deficit significativi nei topi $SG^{+/-}$. Tuttavia, è stato riscontrato un deficit nella preferenza per la novità sociale, in accordo con l'ipotesi di una relazione tra ipofunzione di NMDAR e sintomi negativi di schizofrenia.

Nella successiva serie di esperimenti (Capitolo 4) sono state esaminate le caratteristiche dei topi SynGAP knockout nell'apprendimento strumentale, attraverso

l'analisi dell'inibizione comportamentale e la discriminazione temporale, funzioni note per dipendere dall'integrità del NMDAR. I risultati dimostrano che la mutazione induce un aumento nel vigore con cui un comportamento appreso per condizionamento operante viene eseguito, senza compromettere il controllo temporale, dunque modellando solo in parte i sintomi di disfunzione NMDAR.

Lo studio finale (capitolo 5) ha esaminato l'impatto morfologico e comportamentale della delezione ippocampale di SynGAP, questo fenomeno è associato ad una over-espressione di NMDAR e del recettore per l'acido ammino-metoxy-5-metil-4-isoxazolepropionico (AMPAR), così come a una diminuzione della neuro genesi nel giro dentato dell'ippocampo. Dal punto di vista comportamentale, la delezione ippocampale di SynGAP ha indotto deficit sia nell'acquisizione che nella ritenzione di un compito di memoria spaziale. Inoltre, tale manipolazione risulta influire anche sulla memoria di lavoro e sull'attività locomotoria.

Nel complesso, I presenti risultato forniscono una chiara illustrazione della rilevanza funzionale di SynGAP sulla funzione NMDAR e sul comportamento. Non tutte le funzioni NMDAR dipendenti risultano alterate in seguito alla delezione di SynGAP. Tuttavia, tale modello puo' essere utile nel comprendere i meccanismi NMDAR dipendenti, negli svariati processi fisiopatologici del sistema nervoso centrale, tuttora scarsamente conosciuti.